

**REMARKS**

Entry of the foregoing, reexamination and reconsideration of the subject application, pursuant to and consistent with 37 C.F.R. § 1.116, are respectfully requested in light of the remarks which follow.

**Status**

As is correctly reflected in the Office Action Summary, prior to the instant amendments, Claims 7, 15, 16, 18, 19, 22, and 41-75 were pending. *Final Office Action mailed April 5, 2010, Office Action Summary, Item 4.* Claims 22 and 46-75 have been withdrawn from consideration. *Id. at Item 4a.* Claims 7, 15, 16, 18, 19, and 41-45 stand rejected. *Id. at Item 6.* The Examiner's prior objection to the title and the Examiner's rejection of Claims 18 and 19 under 35 U.S.C. § 112, Second Paragraph, have been withdrawn. *Final Office Action, Page 2.*

**Summary Amendments**

By the foregoing amendments to the claims, Claims 7, 15, 18, 19, and 41 were amended to better clarify Applicants' invention. Claim 22 was canceled without prejudice or disclaimer to Applicants filing one or more continuing applications directed to the previously-presented subject matter.

Claim 7 was amended to specify that the claimed microorganism comprises an *Escherichia coli*-derived NADH-dependent D-lactate dehydrogenase (*ldhA*) gene, wherein the microorganism's FAD-dependent D-lactate dehydrogenase (*dld*) inherent activity is inactivated or decreased, wherein said microorganism's pyruvate formate-

lyase (pfl) inherent activity is inactivated or decreased, and wherein said microorganism's IdhA activity is enhanced. This amendment was clerical in nature and designed to increase readability/comprehension of Claim 7. Support for this amendment may be found throughout the Specification, and at least at [0058] of U.S. Patent Publication No. 2007/0065930, *i.e.*, the published version of the instant application ("the published application"). Accordingly, no new matter has been added.

The amendment to Claim 15 was similarly clerical and similarly intended to increase readability/comprehension of Claim 15. Support for this amendment may be found throughout the Specification, and at least at [0066] of the published application. Accordingly, no new matter has been added.

The amendment to Claim 18 was similarly clerical and similarly intended to increase readability/comprehension of Claim 18. Support for this amendment may be found throughout the Specification, and at least at [0069] of the published application. Accordingly, no new matter has been added.

The amendment to Claim 19 was similarly clerical and similarly intended to increase readability/comprehension of Claim 19. Support for this amendment may be found throughout the Specification, and at least at [0070] of the published application. Accordingly, no new matter has been added.

The amendment to Claim 41 was similarly clerical and similarly intended to increase readability/comprehension of Claim 41. Support for this amendment may be found throughout the Specification, and at least at [0073]-[0074] of the published application. Accordingly, no new matter has been added.

**Rejections Under 35 U.S.C. § 103 – Zhou, Yang, Bunch, and Shaw**

Claims 7, 15, 16, 18, 42, and 44 remain rejected under 35 U.S.C. § 103(a) as purportedly unpatentable over Zhou *et al.*, “*Production of Optically Pure D-Lactic Acid in Mineral Salts Medium by Metabolically Engineered Escherichia coli W3110*,” 69(1) APPLIED AND ENVIRONMENTAL MICROBIOLOGY 399-407 (2003) (“Zhou”) in view of Yang *et al.*, “*Redistribution of Metabolic Fluxes in Escherichia coli with Fermentative Lactate Dehydrogenase Overexpression and Deletion*,” 1 METABOLIC ENGINEERING 141-152 (1999) (“Yang”), as evidenced by Bunch *et al.*, “*The IdhA gene encoding the fermentative lactate dehydrogenase of Escherichia coli*,” 143 MICROBIOLOGY 187-195 (1997) (“Bunch”) and further in view of Shaw *et al.*, “*Vinylglycolate Resistance in Escherichia coli*,” 121(3) J. BACTERIOLOGY 1047-1975 (1975) (“Shaw”). *Final Office Action, Pages 3-9*. These rejections are respectfully traversed.

“A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains.” 35 U.S.C. § 103(a). Underlying factual determinations in an obviousness analysis include (1) the scope and content of the prior art, (2) the level of ordinary skill in the art, (3) the differences between the claimed invention and the prior art, and (4) objective indicia of nonobviousness. *Merck & Co. v. Teva Pharms. USA, Inc.*, 395 F.3d 1364, 1369 (Fed. Cir. 2005) (citing *Graham v. John Deere Co.*, 383 U.S. 1, 17-18 (1966)).

Applicants respectfully submit that a *prima facie* case of obviousness against

Claims 7, 15, 16, 18, 42, and 44 due to Zhou, Yang, Bunch, and Shaw has not been made.

At the outset, it is important to recognize that each of Claims 7, 15, 16, 18, 42, and 44 must be considered as a *whole*. See, e.g., *KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1742 ( 2007) ("What matters is the objective reach of the claim."); *Datascope Corp. v. SMEC, Inc.*, 776 F.2d 320, 324 (Fed. Cir. 1985) (noting that the references must be considered "as a whole" and that the claimed subject matter must be considered "as a whole."). Considering each of Claims 7, 15, 16, 18, 42, and 44 as a whole is important because "a patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art." *KSR*, 127 S. Ct. at 1741. That is because "inventions in most, if not all, instances rely upon building blocks long since uncovered, and claimed discoveries almost of necessity will be combinations of what, in some sense, is already known." *Id.*

Independent Claim 7, as a *whole*, is directed to:

A microorganism comprising an *Escherichia coli*-derived NADH-dependent D-lactate dehydrogenase (*ldhA*) gene wherein said microorganism's FAD-dependent D-lactate dehydrogenase (*dld*) inherent activity is inactivated or decreased, wherein said microorganism's pyruvate formate-lyase (*pfl*) inherent activity is inactivated or decreased, and wherein said microorganism's *ldhA* activity is enhanced.

In issuing obviousness rejections, as opposed to anticipation rejections, the Examiner admits that neither Zhou, Yang, Bunch, nor Shaw alone disclose each and every element of Claim 7. The Examiner also admits that Zhou simply "discloses inactivation of merely *pflB* to generate the SZ32 mutant able to exhibit increased production of D-lactic acid in relation to parental strain W31100." *Final Office Action*,

*Page 4.* Thus, it becomes “important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does.” *KSR*, 127 S. Ct. at 1741.

Applicants respectfully submit that a reason to vary from Zhou has not been provided nor has a sufficient reason been provided to combine *select* aspects of Zhou with *select* aspects of Yang. The Examiner, *without citation to evidence from one of ordinary skill in the art*, states that “by deleting *pfl*, thus reducing or eliminating the routes leading to formation of formate and acetate, the pool of pyruvate would be increased and the flux from pyruvate could be shifted from the production routes of formate and acetyl-CoA to the production route of D-lactate with a reasonable expectation of success.” *Final Office Action, Page 5*. Applicants submit that had Zhou sought to do so, the experiments therein to delete *adhE* and *ackA* would have been unnecessary. Put differently, the entirety to Zhou fails to evidence a desire to do what the Examiner surmises.

Moreover, Yang itself indicates that such predictions were neither certain nor always accurate:

Overexpression of LDH was *expected* to increase the competitiveness of the lactate branch and *might* be able to divert flux away from the acetate synthesis pathway at the pyruvate branch point similar to the heterologous expression of *B. subtilis* acetolactate synthase (Yang et al., 1999). However, the acetate level is *actually higher* for both GJT001 (pTY11) and GJT001(pTY2), which have LDH activities 6 to 8 times higher than that with the control plasmid.

*Yang, Page 146* (emphasis added). Had there been certainty or predictability, the whole stated purpose of Yang – “several strains were constructed to investigate the

competitiveness of various pathways at the pyruvate node. Specifically, the effects of LDH enzymatic activity on the metabolic patterns were examined” – would have been unnecessary. Yang, Page 142. In fact, Yang actually highlights the **complexity** and **absolute unpredictability**:

The positive amplification factors for all three branches *possibly* can be explained by the *highly complicated* regulatory behaviours of the PFL branch. (Yang, Page 150 (emphasis added)).

Taken together, it appears that *in vivo* PFL enzymatic activity not only is influenced by a set of transcription factors, but also is affected by the physiological state of the cell as mediated through a number of metabolites.

The results with overexpression of LDH in the parent strain GJT001 suggest that lactate *may* play a role in the activation of the PFL pathway (Table 6A). ***The effect of the intracellular pyruvate concentration is ambiguous; the PFL branch becomes more active despite decreasing pyruvate concentrations.*** (Yang, Page 150 (emphases added)).

***In summary, the exact activation mechanism in the activation of the PFL pathway is still unclear.*** In particular, the precise role of metabolites in governing the *in vivo* PFL enzymatic activity ***remains to be established.*** (Yang, Page 150 (emphases added)).

The competition among various pathways at the pyruvate node is found to be ***very complicated.*** . . . It illustrates the ***possible complexity*** that may be encountered and the *important role of reaction kinetics (gene activation/repression as well as enzyme kinetics) in our ability to predict and design desired alternative reaction pathways.* . . . This observation illustrates the significance of pathway interactions and ***the need for detailed knowledge*** about overall reaction network dynamics. It also suggests an interesting potential design alternative to increase the flux through a particular pathway not be increasing the enzyme activity of the concerned pathway, but rather by manipulating other part(s) of the network which *may*

have a diminishing effect on the competing branch. (Yang, Page 151, emphases added)).

Put differently, Yang itself acknowledges that the interactions are “complex” and “highly complicated.” The effect of manipulating aspects was “ambiguous.” Exact mechanisms were “still unclear” and there remained a “need for detailed knowledge.”

From at least these expressions of **uncertainty** and **unpredictability** from Yang, Applicants submit that one of ordinary skill in the art would not have modified Zhou and Yang as the Examiner suggests.

With regard to Bunch, the Examiner clarified that “Bunch is merely cited as evidence to the plasmid pFB15, a multicopy vector taught [by] Yang, carrying the *ldhA* gene under the control of the *ldhA* promoter. [The] Bunch disclosure is not part of the claim rejection under 35 U.S.C. 103(a).” *Final Office Action, Page 9*. Accordingly, Applicants respectfully request explicit withdrawal of the mention of Bunch in the rejection of Claims 7, 15, 16, 18, 42, and 44 remain rejected under 35 U.S.C. § 103(a).

Shaw does not cure the deficiencies of Zhou and Yang. Shaw pertains to *Escherichia coli* K-12 vinylglycolate-resistant mutants, including mutants JSH 150 and JSH 151 “lacking both membrane-bound L- and D-lactate dehydrogenases.” *Shaw at Page 1047*. Keep in mind, Shaw published in 1975, i.e., roughly 24 years before Yang. Yet, the uncertainty highlighted above from Yang still existed in 1999. Shaw is *silent* as to requirements of Applicants’ Claims 7, 15, 16, 18, 42, and 44, i.e., that the microorganism has inactivated or decreased FAD-dependent D-lactate dehydrogenase (*ldd*) **and** increased NADH-dependent D-lactate dehydrogenase (*ldhA*) activity. Because Shaw does not distinguish FAD-dependent D-lactate dehydrogenase from

NADH-dependent D-lactate dehydrogenase, Shaw's mutants "lacking both membrane-bound L- and D-lactate dehydrogenases" actually teach away from Applicants' claims' increased NADH-dependent D-lactate dehydrogenase (*ldhA*) activity.

Because Zhou, Yang, and Shaw, either alone or in hindsight-based combination, fail to disclose or suggest Applicants' claimed invention, Applicants respectfully request withdrawal of the rejection of Claims 7, 15, 16, 18, 42, and 44 under 35 U.S.C. § 103(a).

**Rejections Under 35 U.S.C. § 103 – Zhou, Yang, Bunch, Shaw, and Courtright**

Claims 41, 43, and 45 remain rejected under 35 U.S.C. § 103 as purportedly being unpatentable over Zhou in view of Yang, as evidenced by Bunch and Shaw, and further in view of Courtright *et al.*, "*Malate Dehydrogenase Mutants in Escherichia coli K-12*," 102(3) *J. Bacteriol.* 722-728 (1970) ("Courtright"). *Final Office Action, Pages 9-11*. These rejections are respectfully traversed.

The ways in which Zhou, Yang, and Shaw fail to render, *inter alia*, Claim 7 obvious are detailed above. Courtright fails to cure the deficiencies of Zhou, Yang, and Shaw. At best, Courtright pertains to mutants isolated from *Escherichia coli* K-12 who are "devoid of malate dehydrogenase activity." *Courtright, Page 722*. Again, references must be considered "as a whole" and the claimed subject matter must be considered "as a whole." *Datascope, Inc.*, 776 F.2d at 324. Factfinders must avoid the distortion caused by hindsight bias "and must be cautious of arguments reliant upon *ex post* reasoning." *KSR*, 127 S. Ct. at 1742 (citing *Graham* and "warning against a 'temptation to read into the prior art the teachings of the invention in issue' and instructing courts to 'guard against slipping into the use of hindsight.'"). Applicants submit that nothing, other

than Applicants' claimed invention, discloses the subject matter of Claims 41, 43, and 45. Courtright itself is silent as to Claim 41's and Claim 43's requirement that the microorganism's aspartate ammonia-lyase activity is inactivated or decreased.

Moreover, Claims 41, 43, and 45 depend, either directly or indirectly, from independent Claim 7. "If an independent claim is nonobvious under 35 U.S.C. [§] 103, then any claim depending therefrom is nonobvious. *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988)." *Manual of Patent Examining Procedure ("M.P.E.P.")*, § 2143.03. Because Claim 7 is nonobvious, Claims 41, 43, and 45 are similarly nonobvious.

Accordingly, Applicants respectfully request withdrawal of the rejection of Claims 41, 43, and 45 under 35 U.S.C. § 103(a) over Zhou, Yang, Shaw, and Courtright.

#### **Rejections Under 35 U.S.C. § 103 – Zhou, Yang, Bunch, Shaw, and Maier**

Claims 18 and 19 remain rejected under 35 U.S.C. § 103 as purportedly being unpatentable over Zhou, in view of Yang, as evidenced by Bunch and Shaw, and further in view of U.S. Patent Application Serial No. 10/620,487, filed July 16, 2003, published as U.S. Patent Publication No. 2004/0038352, published on February 26, 2004, to Maier ("Maier"). *Final Office Action, Pages 11-12*. These rejections are respectfully traversed.

The ways in which Zhou, Yang, and Shaw fail to render, *inter alia*, Claim 7 obvious are detailed above. Maier fails to cure the deficiencies of Zhou, Yang, and Shaw. At best, Maier pertains to a short list of promoters for increasing expression of a desired gene. See, e.g., *Maier at [0030]-[0031]*. Use of a promoter does not remove the complexity and uncertainty associated with Applicants' invention, as detailed above

with regard to at least Zhou and Yang.

Moreover, references must be considered “as a whole” and the claimed subject matter must be considered “as a whole.” *Datascope, Inc.*, 776 F.2d at 324. Factfinders must avoid the distortion caused by hindsight bias “and must be cautious of arguments reliant upon *ex post* reasoning.” *KSR*, 127 S. Ct. at 1742 (citing *Graham* and “warning against a ‘temptation to read into the prior art the teachings of the invention in issue’ and instructing courts to ‘guard against slipping into the use of hindsight.’”). Applicants submit that nothing, other than Applicants’ claimed invention, discloses the subject matter of Claims 18 and 19. Maier itself is silent as to the particular attributes of Claim 18 and Claim 19.

In addition, Claims 18 and 19 depend, indirectly, from independent Claim 7. “If an independent claim is nonobvious under 35 U.S.C. [§] 103, then any claim depending therefrom is nonobvious. *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988).” *Manual of Patent Examining Procedure (“M.P.E.P.”)*, § 2143.03. Because Claim 7 is nonobvious, Claims 18 and 19 are similarly nonobvious.

Accordingly, Applicants respectfully request withdrawal of the rejection of Claims 18 and 19 under 35 U.S.C. § 103(a) over Zhou, Yang, Shaw, and Maier.

**Rejections Under 35 U.S.C. § 112, Second Paragraph – Purported Indefiniteness**

Claims 7 and 41-45 were rejected under 35 U.S.C. § 112, Second Paragraph, as purportedly indefinite. *Final Office Action, Page 12.* These rejections are respectfully traversed.

Not to acquiesce in the Examiner's rejection, but solely to facilitate prosecution, by the foregoing amendments Applicants have amended Claims 7 and 41 to better clarify Applicants' invention. Applicants believe the foregoing amendments have rendered moot the Examiner's concerns regarding Claims 7 and 41-45.

Accordingly, Applicants respectfully request withdrawal of the rejection of Claims 7 and 41-45 under 35 U.S.C. § 112, Second Paragraph.

**Claim Objections**

Claims 7, 15, 18, and 22 were objected to for reciting "an NADH" instead of "a NADH." These rejections is respectfully traversed.

Not to acquiesce in the Examiner's rejection, but solely to facilitate prosecution, by the foregoing amendments Applicants have amended Claims 7, 15, and 18 to better clarify Applicants' invention. Applicants believe the foregoing amendments have rendered moot the Examiner's concerns regarding Claims 7, 15, and 18. Claim 22 stands withdrawn.

Accordingly, Applicants respectfully request withdrawal of the objections to Claims 7, 15, and 18.

**Request for Rejoinder**

Withdrawn Claims 46-75 are method claims that depend, directly or indirectly, from allowable independent Claim 7. Pursuant to at least M.P.E.P. § 821.04, Applicants respectfully request that Claims 46-75 be rejoined with allowable Claims 7, 15, 16, 18, 19, and 41-45.

**CONCLUSION**

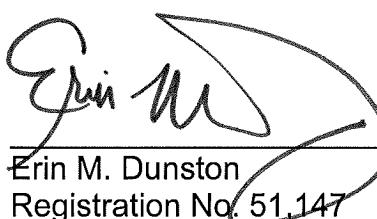
In the event that there are any questions relating to this Amendment and Reply After Final Rejection Pursuant To 37 C.F.R. § 1.116, or to the application in general, it would be appreciated if the Examiner would contact the undersigned attorney by telephone at (703) 836-6620 so that prosecution of the application may be expedited.

The Patent Office is hereby authorized to charge any necessary fees, or credit any overpayment, to Deposit Account No. 02-4800.

Respectfully submitted,  
BUCHANAN INGERSOLL & ROONEY PC

Date: July 22, 2010

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